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BASEL (Schweiz) S. KARGER NEW YORK

Separatum *Vol. 6, No. 4/6* Printed in Switzerland

Transactions of the Collegium Internationale Allergologicum
First Symposium in London, October 12th—16th, 1954

Paton, W. D. M.: Int. Arch. Allergy *6*, 203—229, 1955.

Histamine Metabolism

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The Metabolism of Histamine

This paper will attempt to discuss the metabolism of histamine in its broadest aspects, surveying the whole life of histamine in the body. One can put four questions:

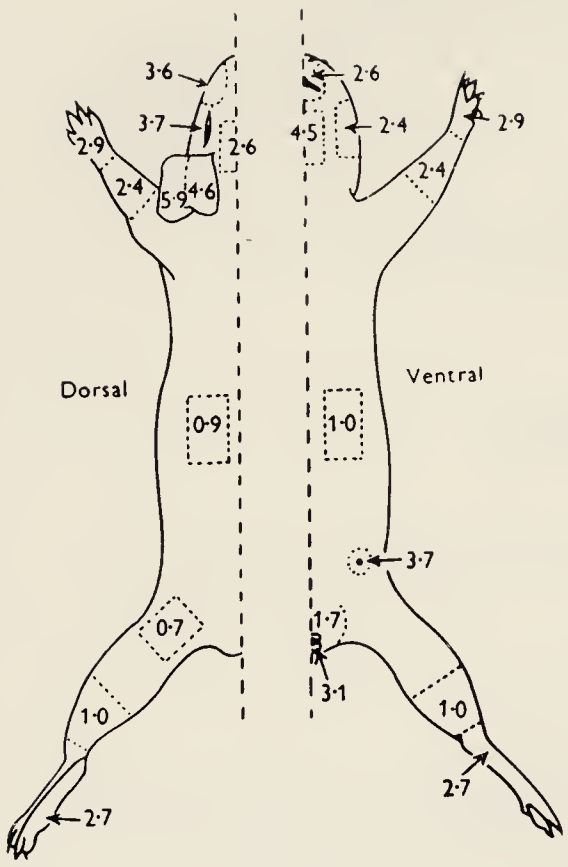
1. Where is histamine located?
2. Where does it come from?
3. How is it set free?
4. What is its fate when it is mobilized?

Although we have only imperfect answers to all these questions, yet it is only when we can answer them that anything like a satisfactory account of the metabolism of histamine can be given.

Where is the Histamine?

There have now been a good many studies on the distribution of histamine. Most of these have used the relatively crude method of taking a portion of a particular tissue, grinding it up in some acid, possibly extracting it further (to exclude other pharmacologically active materials) neutralizing, and finally assaying on some test object such as the guinea-pig ileum. When this is done, one can describe the gross distribution of histamine somewhat in this way.

First, it is present throughout the *skin*, sometimes in remarkably large concentration; thus in the skin of the cat's ear (*Smith*, 1953) or in the tip of a mouse's tail there may be nearly 100 μg/g. On the other hand, other areas of the skin such as the flanks, or inner aspects of limbs, may have only relatively small amounts, 2–10 μg/gm. The distribution of the higher concentrations seems, in general, to be in those parts of the body, such as face and paws,



← *Fig. 1.* Histamine content of guinea-pig skin: abdominal skin taken as unity. (From *Feldberg* and *Miles*, 1953.)

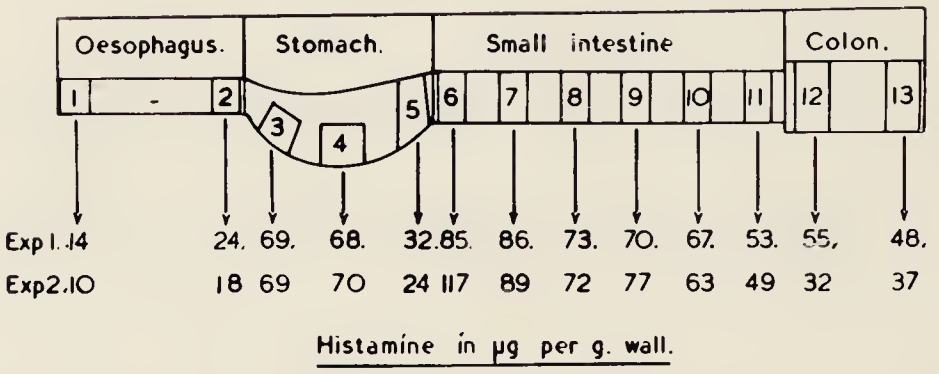


Fig. 2. Histamine content of dog's alimentary tract (µg per g wall). (From *Douglas* et al., 1951.)

that come most readily into contact with the outside objects (cf. *Feldberg* and *Miles*, 1953).

The second important location of histamine is in the *intestinal tract*, and here it is found in the whole extent of the intestine. But again, it is not uniformly distributed here. There are local variations within the stomach, and there is a general tendency for the content to decrease as one moves down the intestine from the head towards the tail, so that the colon is relatively poor in it (*Douglas* et al., 1951).

A special site, associated with the alimentary tract, is the *liver*. Here we encounter a very marked species variation. In the dog and rat for instance, there is a considerable amount of histamine, whereas in the cat, guinea-pig, and human, there are only small amounts.

The third major site of histamine is the *lung:* in all species, high values are found.

Finally, we may take the other structures in the body as a rather heterogeneous group, possessing significant, but never containing outstandingly high amounts. *Muscle* has only low content; *brain* a trivial amount, except in the hypothalamic region, where *Harris*, *Jacobsohn* and *Kahlson* (1952) have been tempted to suggest it plays some role in initiating pituitary activity. The *uterus* usually has a moderate content (10–20 µg/gm), unless it is pregnant, when it falls to vanishing point (*Gunther* and *Paton*, unpublished). *Spleen*, *pancreas*, *kidney*, *submaxillary gland*, *parotid gland*, *thyroid gland*, *adrenal*, and *heart*, all have moderate or low values. The formed elements

of the blood often contain histamine. The *polymorphs* contain it in humans. The *platelets* contain histamine in rabbit, goat, guinea-pig and cat, but not in dog, rat or man (*Humphrey* and *Jaques*, 1954).

Reviewing this work, one generalization suggests itself: the regions consistently rich in histamine, viz. skin, intestine and lung, are the surfaces at which the organism meets the outside world. Further, in the skin, it is those regions most frequently in such contact (paws, nose, ears, eyelids) that are richest.

So much for a general macroscopic study. The next question is how histamine is distributed on a finer scale. There have been three main developments in this direction. First, we have studies in which a tissue is dissected into its component parts, or is sectioned by a microtome. We then find that, for instance, in the intestine the mucosa may contain strikingly and consistently high amounts, although the external muscles of the intestine regularly contain much less (*Douglas* et al., 1951). Similarly, in the skin, the epidermis may be richest and the deeper layers much less (*Harris*, 1927).

An important observation made in recent work shows that *mast-cells*, already known from the work by *Wilander* and *Jorpes* to contain heparin, also have substantial amounts of histamine. *Riley* and *West* (1953) have shown that there is a significant correlation between mast-cell incidence and histamine content in the capsules of liver and lung, and in mast cell tumours, and under conditions where histamine content varies with age or after histamine release. This means of course that the places where mast-cells are prolific will also be rich in histamine.

But the distribution of histamine has been followed even further and has been shown to be present (using a tissue such as liver or lung), in the intracellular particles of the cell, chiefly the mitochondria, rather than in the microsomes, the cytoplasm, the nucleus, or attached to the cell membrane (*Hagen*, 1954; *Copenhaver* et al., 1953; *Mongar* and *Schild*, 1954). We have to think, therefore, of a good deal of the histamine in the body as located either in granular form in mast-cells or in minute localizations of histamine within liver and lung cells. It is doubtful whether this accounts for the state of all the histamine in the body, such as for instance that in the skin epidermis, or that in the small intestine. Here there may well be yet a third form, in which histamine is bound so that it is present, but pharmacologically inactive.

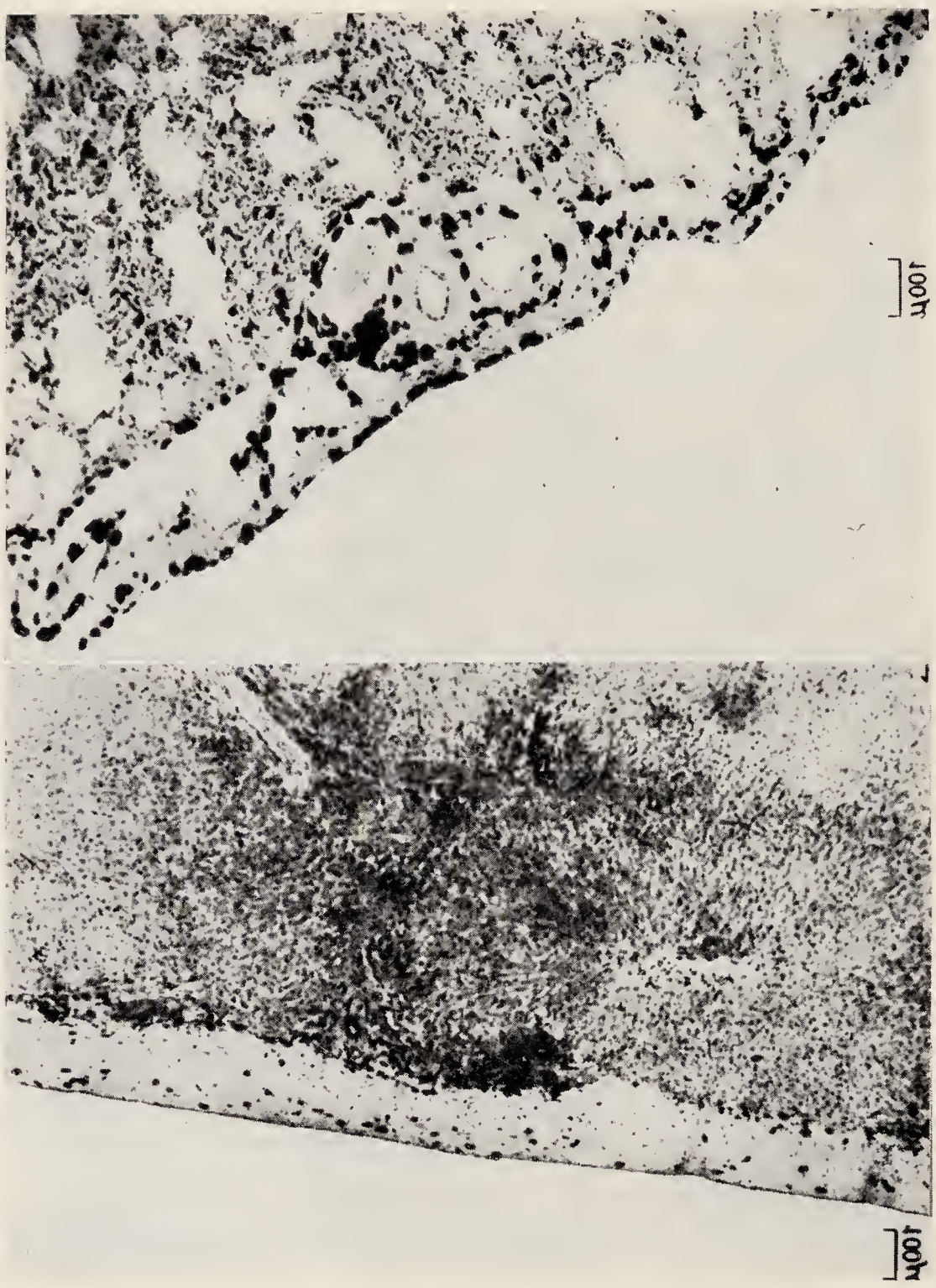


Fig. 3. Microscopic sections of ox lung and ox liver, showing mast-cells in pleura and liver capsule stained with toluidine blue. (From *Riley* and *West*, 1953.)

Where does Histamine come from?

The first obvious source of histamine would be that it is ingested in the food and no doubt there is some in an ordinary diet. But only small amounts could enter the intestinal tract in this way. Milk contains $< .5$ μg/gm, bread $< .1$ μg/gm, and meat about .5 μg/gm (*Anrep* et al., 1944; *Hardwick*, 1954). Bearing in mind that of histamine orally administered only about 5 % can be accounted for in the urine as histamine or acetyl-histamine, this means that only trivial amounts can enter from this source.

A more important source is the histamine produced within the intestine through the action of bacteria. A number of organisms of the coli group are able to decarboxylate histadine and so produce free histamine in the intestinal-lumen. To them, no doubt, is due the quite large amount of histamine here, up to 10 mgm in human faces, and up to 5 mgm in the caecum (*Hanke* and *Koessler*, 1924); there are similar, or proportionately bigger quantities in the dog. But a good deal can happen to histamine before it appears free in the blood after it appears in the lumen. Firstly, there are also organisms which can acetylate histamine in the intestine, so that it is no longer absorbed in an active form (*Urbach*, 1949). Secondly, if histamine is given to an animal in a large dose, a good deal of it apparently gets bound in the intestine at two points – firstly to the gastric or intestinal juice, and secondly in the intestinal wall (*Parrot*, 1949). Finally there is in the wall of the intestine a considerable quantity of histaminase, which destroys the histamine passing through the cells (see *Lindell* and *Westling*, 1953, for references). The net result of this is that histamine given by mouth or formed in the intestine produces a relatively tiny rise in the free histamine level in the blood, although it can cause a more easily detectable rise in urinary histamine (*Hanke* and *Koessler*, 1924; *Anrep* et al., 1944). It is possible, nevertheless, to produce what may be important changes in the gut (and as a result in the urinary) histamine by influencing the bacterial state of the intestine, and a rather interesting sequence of events is seen. If a broad range antibiotic is given, the bacterial flora are depressed, and the amount of histamine entering the body is reduced (as measured by the excretion of histamine in the urine). When the antibiotic is removed, the histamine entering the body not only returns to normal, but may actually overshoot to as much as a hundred per cent above the original value for a period of time corresponding roughly to that required for normal intestinal flora to re-establish themselves (*Wilson*, 1954).

It is unlikely, however, that the source of all or even most of the body histamine is from the intestine. If an animal is starved (*Haeger* and *Kahlson*, 1952), there is no effect found on the histamine content of the intestinal tract. Nor does treatment with antibiotics influence the tissue histamine except for some reduction in the content of the small intestine, a not improbable result in any case – as it is here that histamine is absorbed. It seems probable therefore

that decarboxylation of histidine can take place in the body independently of bacterial action; a supposition which follows at once from the known presence of the appropriate decarboxylase in the liver and kidney, and sometimes in pancreas (see *Blaschko*, 1945, for references), implying that the amine can be formed at these sites at any rate.

There are some puzzles about the source of histamine. First, one curious fact about histamine rests in some experiments by *Dekanski* (1945) in which he found that a mouse after being burnt developed more histamine in the body, so that the whole body 10 minutes after burning contained nearly twice as much as there was to begin with. In other words, there had been, not a mere transport of histamine from one region to another, but an actual creation of new histamine; this creation could not be attributed to bacterial action, since the histamine in the intestine was unchanged. Where this histamine came from is very obscure. It is known that burns activate protease in the skin, and it may be that the histidine was released by the protease and this was then decarboxylated to histamine. But there is really no decisive evidence on the point. The puzzle is this: how can an amount of histamine equal to all that in the body be synthesised in 10 minutes, when we know that after exposure to histamine liberators prolonged reductions of tissue histamine are seen?

Second is the sometimes rather striking change of histamine content with age and size. This was first studied by *Trethewie* but has now been examined by a number of other workers. The clearest fact is that the foetal animal is very poor in histamine, but accumulates it as birth approaches, reaching (in guinea-pig and rat at least) fairly high levels at birth. (*Trethewie*, 1947; *Misrahy*, 1946; *Hardwick*, 1954). Thereafter there seems to be some decline in histamine content of lung and skin, until a rather dramatic rise at the time of weaning in the rat. This declines, and there then appears to take place, but possibly only in large animals, a slow increase in histamine content, marked, however, by steadily greater individual variations. Those who perfuse cat's muscle or skin or lung, for instance, gain the impression that it is with larger and older cats that high histamine contents and outputs are obtained. An interesting comment is that by *Misrahy* that the histamine content in the fetus is more or less uniform, and that it is only in adult life that differentiation develops.

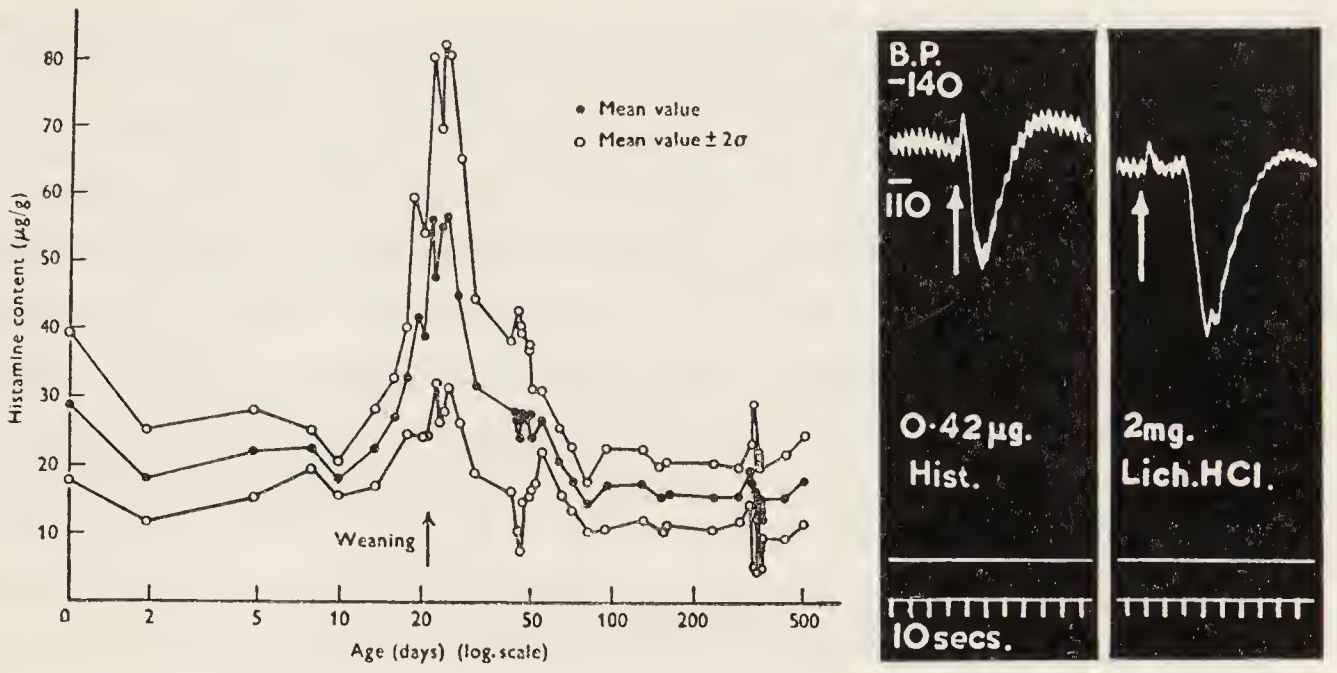


Fig. 4. Histamine content of rat skin against age from birth. (From *Hardwick*, 1954.)
Fig. 5. Depressor responses, in the chloralose cat, to injections of histamine and of the histamine-liberator licheniformin, showing the "delayed depressor response". (From *MacIntosh* and *Paton*, 1949.)

Viewing this general tendency for histamine content of tissues to increase and differentiate with general experience of the world, together with the facts of its distribution in the adult animal – principally at the interfaces between the animal and the world – (the skin, the lung, and the intestine) – one is tempted to indulge the "higher speculation", and to suggest that it is the contact with the outside world, the minor knocks and bumps of life, whether physical or chemical, that determine the histamine content of a tissue. It is, in fact, known, from *Dekanski*'s work that such insults to the skin as burning at 60° C., or injecting leukotaxine, will cause very considerable rises in skin histamine: some of this might be due, indeed, to localization and breakdown of platelets or leucocytes at the site of treatment. But remembering *Dekanski*'s work already mentioned, that extensive burns in mice cause the total histamine extractable from the mouse to increase, it is obvious that more than a mere redistribution of histamine may be involved.

The result of this speculation, then, is firstly to present the high histamine content of a tissue as a sort of label that this tissue is on the frontier between the organism and the outside world: and if one believes histamine to be functionless, one could go no further. But probably few people in fact believe that histamine has no purpose in the body, but would suggest that this purpose is still undiscovered. A possible function is suggested if one turns, for in-

stance, to some old experiments by *Evans* et al. (1948) who found that the pathogenicity of staphylococci and other organisms in skin was enormously enhanced by inoculating them together with adrenaline, so that the invasion started in an ischaemic region. It would be interesting to know whether histamine would, by capillary dilation, have reduced their pathogenicity. Tiresome as histamine is, one could suggest, then, that it is to prevent favourable ischaemic conditions for bacterial invasion, that the body's surfaces contain histamine, and (when assaulted) replenish their stocks even more fully.

How is Histamine Mobilized?

Ten years ago the substances known to cause the release of histamine from animal tissues were relatively few, principally owed to *Feldberg* and *Rocha e Silva* (see *Feldberg*, 1941, for references). They included venoms, proteases, lecithin, certain bacterial toxins, peptone, and anaphylactogenic drugs; none of these agents were of known chemical structure and their action seemed to depend on direct tissue damage. Only two specific compounds were suspected of causing histamine release – d-tubocurarine from muscle (*Alam* et al., 1939), and adrenaline, which *Eichler* and *Barfuss* (1940) claimed could, in man, cause some elevation of the blood histamine.

In 1947 *Schild* and *Gregory* confirmed unequivocally the observation that d-tubocurarine could release histamine from skeletal muscle and showed that strychnine could also do this. In the same year, *MacIntosh* and *Paton* (1947 and 1949) showed that the ability to release histamine was far commoner than previously suspected. It was exerted by diamines, diamidines, diguanidines, di-isothioureas and diquaternary salts as well as a number of benzamidine derivatives and a number of other substances with slightly more complicated molecules. All this may be summarised by saying that there was added to the group of substances known to release histamine approximately 70 specific chemical compounds, all bases and many of them of a very simple dipolar structure.

Since that time the number of substances releasing histamine from tissues has rapidly multiplied and the situation is now somewhat confused, both as to the mechanism of release with many of the substances in question, the effects in different species, and the relationship to the phenomenon of sensitisation. But the property has now been recorded so often, and the process of histamine

release presents, in some cases at any rate, so characteristic a picture that it seems desirable to review the situation at present and to try to disentangle the main trends. We shall start by describing the properties of what will here be called a "histamine liberator" to distinguish it within the general class of histamine releasers.

The Properties of Histamine Liberators

The delayed depressor response

The phenomenon which first attracted *MacIntosh* and *Paton*'s attention was the delayed depressor response in the cat to an injection of the antibiotic licheniformin. If a substance such as histamine or acetylcholine is injected into the circulation of a cat anaesthetised with chloralose there occurs after a latency of 5–8 seconds a rapid and transient fall in blood pressure. With licheniformin on the other hand there was a delay of about 20 seconds during which there was no effect whatever in the B. P.; then it fell as abruptly as with an injection of histamine or acetylcholine and often (with small doses) recovered nearly as quickly. This was a very suggestive phenomenon, for it showed that the substance had itself no immediate effect on the heart or the blood vessels (since this would have manifested itself during the first 20 seconds). It also indicated that it was not having a direct delayed action of its own, because when the fall in B. P. appeared it was at least as rapid as with histamine or acetylcholine itself. The magnitude of the latency is also significant and suggested that it corresponded in fact to the circulation time, a presumption which *Gray* and *Paton* (1949) later found to be correct. These considerations led to the hypothesis that the drug was acting in an indirect manner by passing to the tissues, releasing there some vaso-active material which then returned to the heart and during the next circulation of blood exerted its effect. Since histamine is known to exist in considerable quantities in most of the tissues of the body, and since there is no enzyme in the blood able to destroy it with extreme rapidity (as there is for acetylcholine,)histamine was an obvious candidate for the vasoactive principle and it proved in fact to be the substance released.

The proof of this rested on the following experiments:

1. After the injection of a large dose of licheniformin the plasma of the recipient animal acquired a depressor activity of

short latency which survived the extraction procedure of *Code* and was antagonised by anti-histamines. When tested on the guinea-pig's ileum the histamine equivalent of the plasma before and after a *Code* extraction agreed with that obtained from the cat's B. P.; and in this preparation also, it was antagonised by anti-histamines but not by atropine.

2. It is characteristic of histamine that, in a cat with a low blood pressure, it often provokes a secretion of adrenaline, so that the initial depressor action is followed by a brisk tachycardia and pressor response. In animals in which histamine produced this effect licheniformin did also.

3. Histamine characteristically causes (in fairly large doses) haemo-concentration and an increase in the volume of limb. The histamine liberators also do this.

4. Histamine is able to elicit gastric secretion; licheniformin was also found to do this.

5. Licheniformin, like histamine, produced a typical triple response when injected intradermally into human skin.

6. A consideration of the amounts of histamine released indicated that they were adequate to account for the effect observed.

It was concluded from this evidence that licheniformin, together with the large number of other bases which also produced the delayed depressor response (for representative members of which similar evidence was obtained), acted by the release of histamine from the tissues with which they came in contact, through the circulation. With many of the compounds, though not all, this property of histamine liberation accounted for all their pharmacological action. For purposes of research, using a histamine-liberator, Compound 48/80 is probably the most generally suitable, from its high potency and specificity (*Paton*, 1951).

The Release of Heparin and Slow Reacting Substances

The release of histamine, as has already been stated, accounted for the main circulatory effects seen. But in the dog it was also noticed that the blood, following the injection of a histamine liberator, became incoagulable. This defect of coagulation could be reversed by toluidine blue and some evidence was obtained that

the plasma could produce with toluidine blue the characteristic metachromatic reaction seen with heparin. Although heparin has never been isolated as such from a dog shocked with a histamine liberator, it seems highly probable that it is heparin which is in fact released, and it will be referred to as such.

In addition to heparin there appears in the blood in many experiments a "slow reacting substance" (S. R. S.). This can be detected on the guinea-pig's ileum after the histamine has been antagonised by mepyramine. The latter drug abolishes the quick contractions produced by histamine and allows there to be revealed, when larger doses of plasma are added to the bath, a much more slowly developing contraction of the intestine.

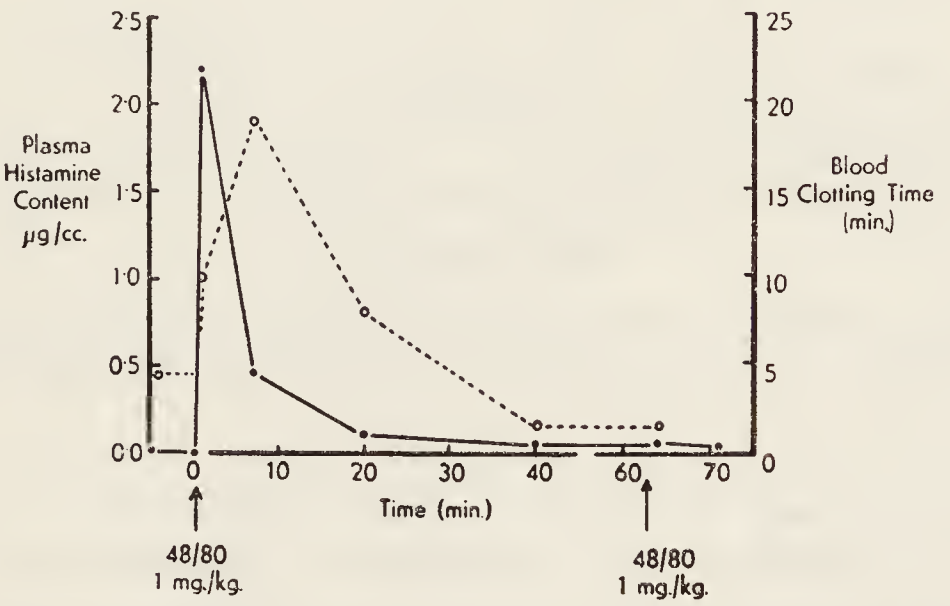


Fig. 6. Time course of rise in plasma histamine and in clotting time of blood, in a chloralose dog after intravenous injections of 1 mg/kg. Compound 48/80. (From *Paton*, 1951.)

It is therefore slightly misleading to refer to these compounds simply as histamine liberators, since they are capable of liberating at least two other substances into the circulation. An interesting point is that the release of these three materials run different time courses. The concentration of histamine in the plasma is at its maximum within 1–2 minutes of the injection. The defect of coagulation is at its maximum 5–10 minutes later, and the plasma content of S. R. S. is usually at its height later still. This need not indicate three separate processes, but may well be a reflection of the diffusibility of the three molecules; that of histamine is the smallest, of heparin perhaps the next, and of S. R. S. (if one accepts the presumption that it is a polypeptide) largest of all.

The Site of Histamine Release

In the cat, *MacIntosh* and *Paton* produced evidence that the release did not take place from the lungs or viscera, that an injection into the lower part of the aorta was more effective than an intravenous injection, and that flaying the animal did not abolish the response. They concluded on this circumstantial evidence that in

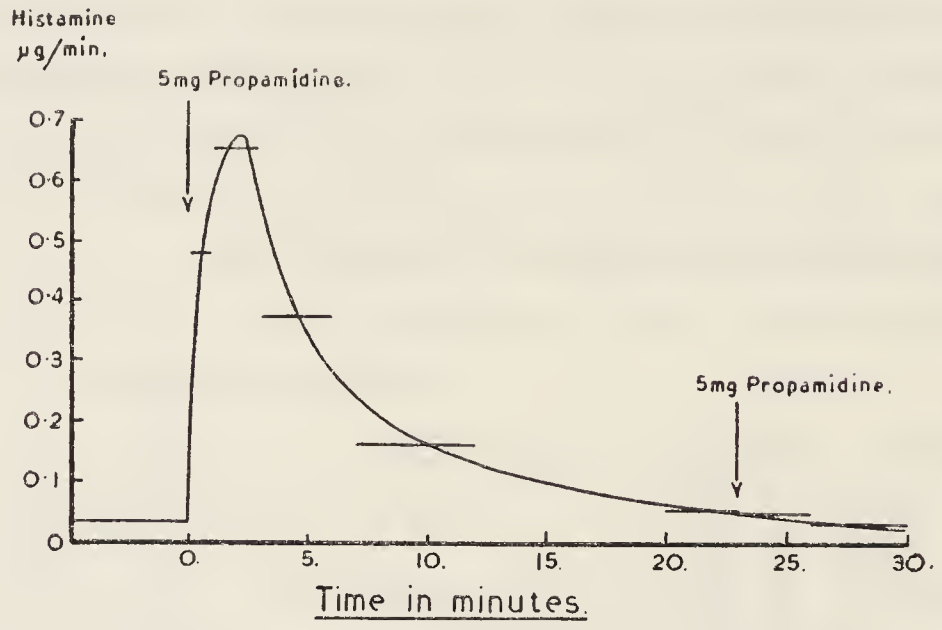


Fig. 7. Release of histamine from isolated perfused cat's gastrocnemius, after arterial injection of 5 mg propamidine. (From *Feldberg* and *Paton*, 1951.)

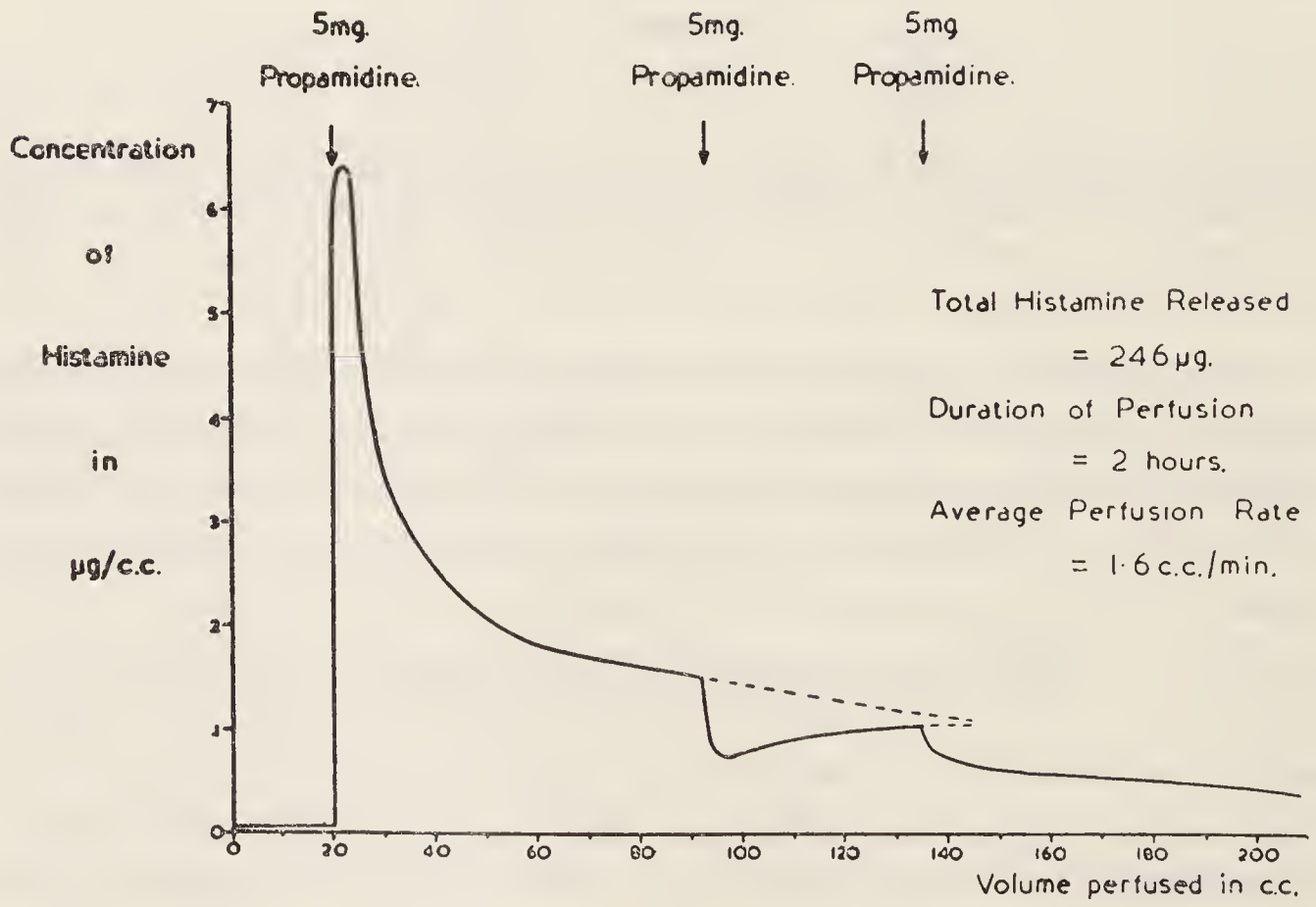
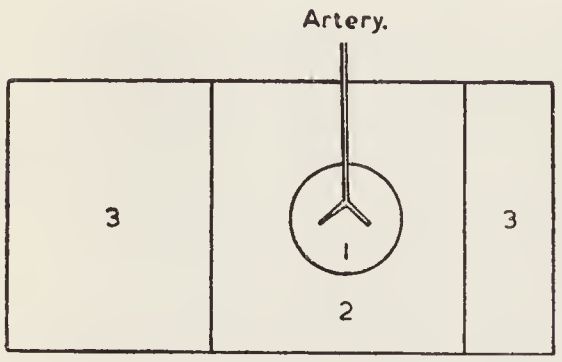


Fig. 8. Release of histamine from isolated perfused skin of cat's leg, after intraarterial injections of propamidine. (From *Feldberg* and *Paton*, 1951.)

the whole animal it must be the tissues such as constitute the mass of the lower part of the body, i. e. both skin and muscle, in which histamine release takes place. Direct evidence for this was obtained by *Feldberg* and *Paton* (1951) using isolated perfused preparations of skin and muscle. It was found that much more histamine was released from the skin than from the muscle, for two main reasons.



Skin sample	Histamine content after injection of Propamidine
1	0·3 µg./gm.
2	1·5 µg./gm.
3	10 µg./gm.
Control	15 µg./gm.

Fig. 9. Diagram showing how the central part of a piece of isolated perfused skin can be almost completely depleted of histamine by a histamine liberator. (From *Feldberg* and *Paton*, 1951.)

The first was that the histamine content of the skin could be completely exhausted, whereas not more than $^2/_3$ and often less of the histamine in the muscle could be released. The second reason was simply that the amount of histamine in the skin is normally much greater than in muscle, sometimes ten times as great.

In the dog the liver plays a dominant part. After the injection of a histamine liberator intravenously the portal pressure rises and the liver can be seen to swell and becomes turgid to palpation. If the injections of the liberator are made intraportally, the effect produced is greatly enhanced. From these facts it is clear that a substantial proportion of the release takes place from the liver. This fits with the appearance of heparin in blood of the dog, since this is also probably a hepatic effect. *Feldberg* and *Schachter* (1952) have shown that histamine liberators will also release histamine from the isolated perfused skin of the dog, and verified that isolated skin in vascular continuity with the animal is also susceptible (a fact already suspected from the pronounced oedema and itching which *Paton* and *Schachter* (1951) observed after injecting 48/80). Perfused intestine and lung, despite the high content of histamine, did not release it.

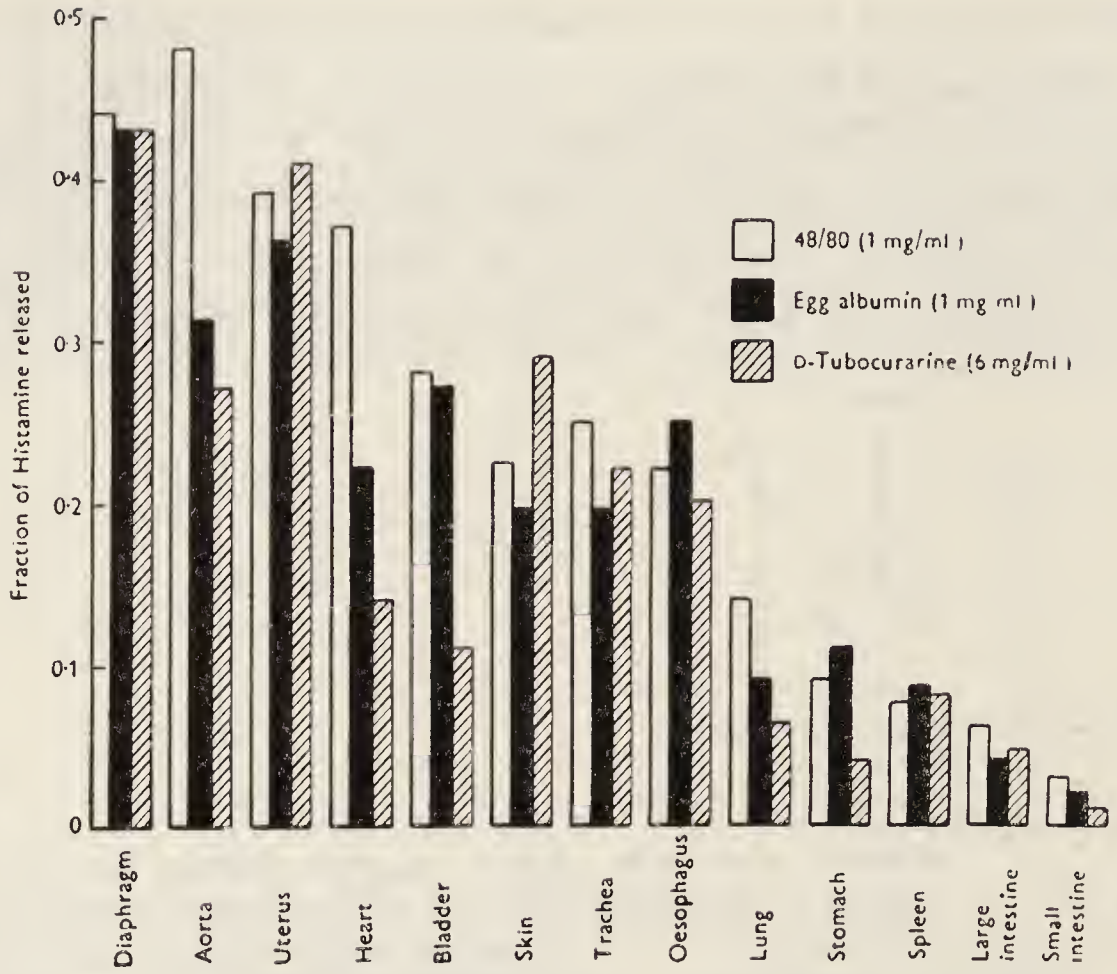


Fig. 10. Comparison of the histamine release by 48/80, antigen, and d-tubocurarine on various guinea-pig tissues *in vitro*. (The animal had been sensitized to egg-albumen). (From *Mongar* and *Schild*, 1952.)

Experiments on other tissues have been done, not in the whole animal or with the whole perfused organ, but using the diffusion technique developed by *Schild*. With this it was found that histamine liberators in fairly high concentration could release *some* histamine from almost all tissues, although there are puzzling differences (*Mongar* and *Schild*, 1952).

Refractoriness

If a histamine liberator owes its effects to the release of histamine normally stored in inactive form in the tissues, then its effects should gradually diminish with repeated injections, as the store of available histamine diminishes. There are now numerous experiments which can be interpreted in this way. First, *MacIntosh* and *Paton* found that successive doses of licheniformin and other liberators produced steadily less and less effect on the B. P. the more frequently effective doses were given. After a big dose it may indeed be quite hard to obtain a response again, even if one waits for several hours. Second, they found that if an intradermal injec-

tion was made at the site of a previous injection of a liberator, but between 6 and 24 hours later, it no longer produced its effect, even though histamine itself could still elicit a wheal and even though the liberator injected at a place where histamine had previously been effective was still active. In other words, the skin

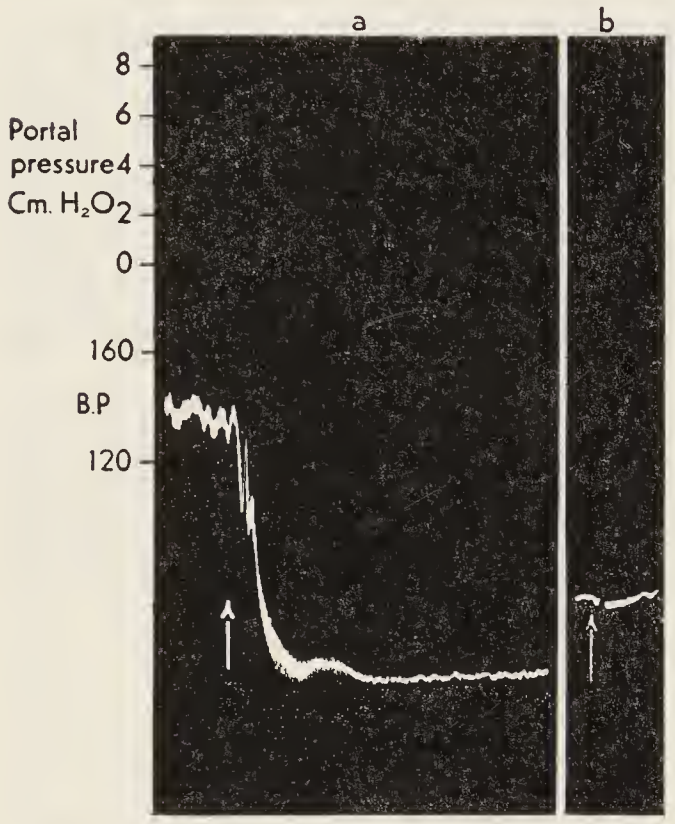


Fig. 11. Effect of two injections of Compound 48/80, 1 mg/kg, on the blood pressure and portal venous pressure of a dog anaesthetized with chloralose, showing the ineffectiveness of the second injection. (From *Paton*, 1951.)

after an injection of the liberator is refractory to the production of a wheal for 1–2 days afterwards. This is precisely analogous to some observations by *Grant*, *Pearson* and *Comeau* (1935) on emotional urticaria. Third, in the dog it was found that after a previous large dose of a liberator the rise in portal pressure in plasma histamine

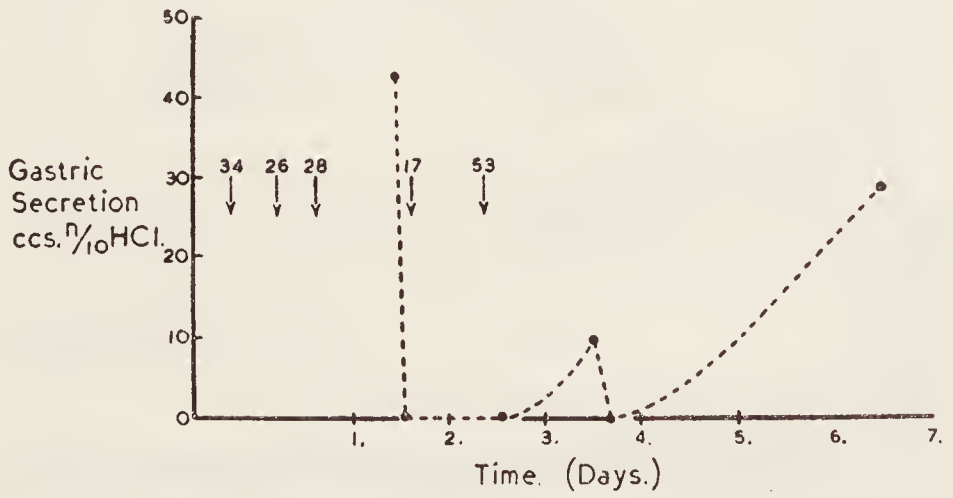


Fig. 12. Effect of repeated injections of 48/80 (10 mg) at varying intervals on the acid gastric secretion of the unanaesthetized dog (gastric fistula). Inserted figures are the response (ccs N/10.HCl to 1 mg histamine acid phosphate ryInsemes marked by the accompanying arrows. (From *Paton* and *Schachter*, 1951.)

and in coagulation time previously seen could no longer be elicited. Fourth, in the dog with a gastric fistula the gastric secretion induced by the injection of a liberator intravenously is very much smaller if the injection took place 1–2 days after an earlier injection, although the stomach could still produce a vigorous secretion (even possibly somewhat augmented) to histamine itself (*Paton* and *Schachter*, 1951). Fifth, rats which have received an injection of liberator, responding to it with the characteristic oedema of the paws and face do not show this to a subsequent injection. With repeated treatment indeed *Feldberg* and *Talesnik* (1935) showed that the skin of the abdomen of the rat may become completely depleted of histamine and fail to return to normal for very many days.

It is clear therefore that, as one would expect, the release of histamine is followed by a period of refractoriness to further release which can be attributed in part at least to the exhaustion of the

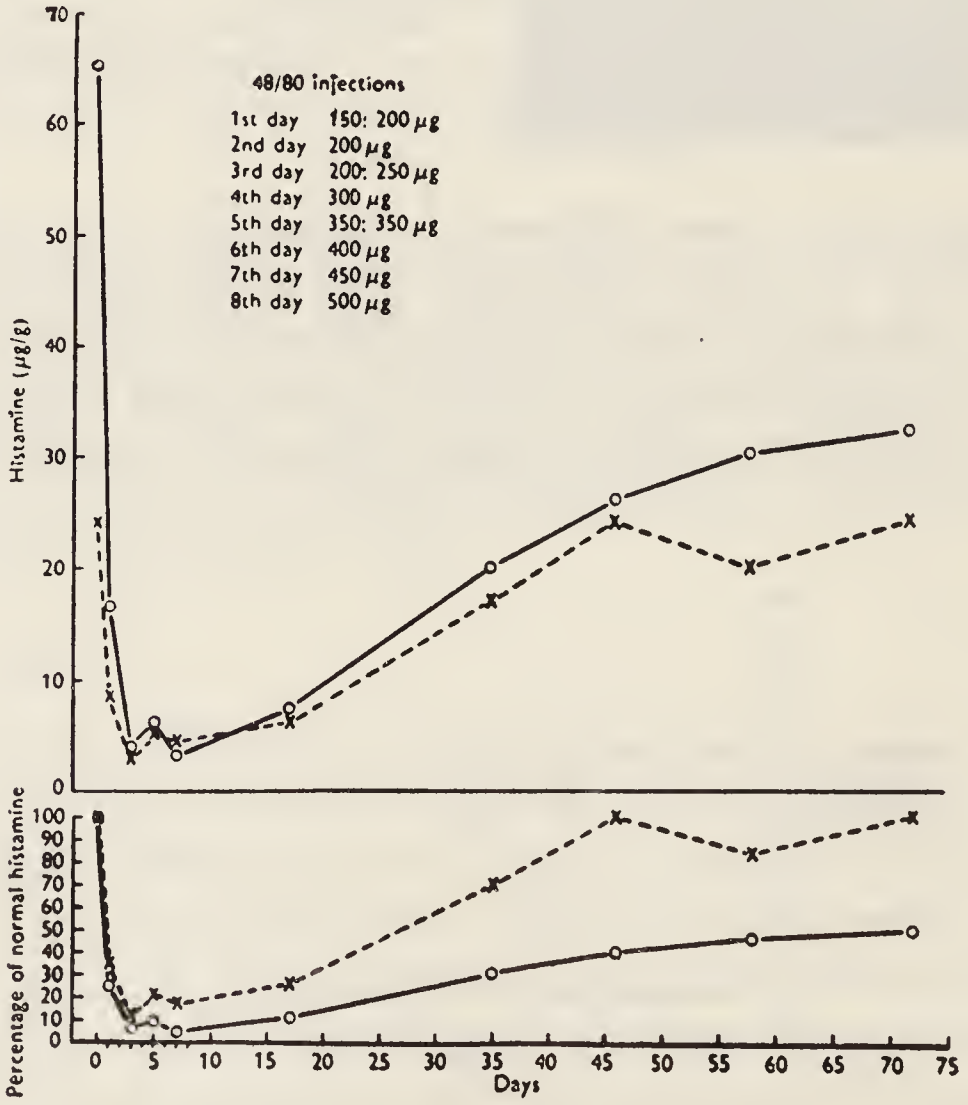


Fig. 13. Effect of intraperitoneal injections of 48/80 on histamine content of rat's skin from abdomen (×---×) and feet (O—O). Upper tracings histamine content expressed in μg/g skin; lower tracings in percentages of normal values. Eight days' treatment with increasing doses of 48/80 as indicated. (From *Feldberg* and *Talesnik*, 1953.)

available histamine. An interesting outcome of this type of investigation is the light it throws on the slowness of the re-synthesis of the histamine in the skin.

Effects of Histamine Liberators in Man

The most extensive experience of this is to be found in the use of the therapeutic diamidines for the treatment of tryponosomiasis. Vivid descriptions of the effect of an injection of one of these are furnished in some clinical reports (*Kirk* and *Henry*, 1944; *Lourie*, 1942). The natives receiving the injection danced around, shouting "Niml! Niml!" meaning "Ants! Ants!", and trying to scratch themselves all over at once. Their antics greatly delighted their friends, who regarded it as proof that a really good medicine had been administered. Along with these effects on the skin was sometimes observed a pronounced fall in blood-pressure, colic and diarrhoea. It seems from these results that histamine release from human skin is readily achieved by histamine liberators circulating in the blood. There is abundant evidence of course (some already mentioned) that these compounds when injected directly into the skin intradermally will release the histamine there.

Fig. 14. *Some substances in human use which can release histamine.*

D-tubocurarine Laudexium Mytolon Succinylcholine	Quaternary Salts	Propamidine Pentamidine Phenamidine Stilbamidine Antrycide	Chemotherapeutic agents
Morphine Codeine Papaverine Thebaine Pethidine Atropine Strychnine	Centrally active compounds	Arfonad Apresoline Priscol	Depressor drugs
		Amphetamine Tyramine Phenylethylamine	Sympathomimetic agents

Lobster, mussel & crayfish extracts
Protein hydrolysates

A second reaction to be attributed to histamine release is the *nitritoid crisis*, a grossly unsuitable name, which should be replaced by a more appropriate term such as "anaphylactoid". Among the agents which will produce this are the opium alkaloids which are known to be true histamine liberators. The crises are characterised

by falls in the blood-pressure, more or less extensive urticaria, sometimes oedema of the mucous membranes, and sometimes collapse with peripheral circulatory failure. These appear, in short, to be rather more severe forms of the reaction described above with the therapeutic diamidines.

Thirdly, we have the production of bronchospasm by known histamine liberators. The observation that morphine belongs to this group (*Feldberg* and *Paton*, 1951) explains the well known clinical danger of giving morphine in bronchial asthma, when it often leads to a fatal outcome. Similarly, it is known that in a small (a very small) proportion of patients d-tubocurarine can produce bronchospasm, although muscle relaxants with less histamine liberating activity do so less frequently.

From these gleanings, it is clear that man certainly resembles the cat in being able to release histamine from his skin, and the guinea pig in incurring bronchospasm. There is not sufficient evidence to know whether heparin release takes place as in the dog.

Modes of Histamine Release and Classification of the Active Drugs

So far the account has been deliberately restricted to those drugs which are known to produce a delayed depressor response on the cat's blood-pressure, a wheal in human skin, or release of histamine from perfused cat's skin. These compounds have been referred to as histamine liberators in the belief that they exert a specific action and do not operate either by tissue damage or through sensitisation reactions. One might concoct a working definition of these as "bases, causing on first administration the release of histamine into the blood of cats and dogs, when given intravenously, or from the skin of cat, dog and man, as well as other species, when injected into the skin, with a roughly uniform potency by each test, without causing acute tissue damage, and also capable of histamine release in other tissues." But there are many other substances which must now be considered which are also capable under various conditions of causing histamine release.

1. *Substances causing acute tissue damage or irritation.* These include proteases, snake venoms, lysolecithin and bacterial toxins.

2. *Substances producing sensitisation*, in which previous exposure to the drug in question is necessary. These include all the foreign proteins and things that can unite with proteins. It is possible that

the interesting release of histamine by neoarsphenamine recently described by *Schachter* (1952) belongs in practice to this group since it is known to be capable of coupling with proteins, and the "nitritoid" reactions to it do not appear until 1 or 2 injections have been made. It does not produce a delayed depressor response in the cat, but it can be made to release a small amount of histamine from the perfused cat's skin.

3. *Surface active substances.* The surface active compound Tween 20 is known to produce gross urticaria and itching in the dog, together with incoagulability of the blood (*Krantz* et al., 1948). *Schachter* (1952) has shown that bile salts can release histamine from the perfused skin and suggests that release of this sort contributes to the pruritus of obstructive jaundice. It is obviously possible that these substances work in quite a different way from the histamine liberators, since they might well produce lysis of the membranes of the cells containing histamine. When injected into the cat, surface-active materials of this sort produce an immediate depressor response unlike that due to either histamine or histamine liberators, and possibly due to direct depression of the heart's beat, and they are far from active in releasing histamine from intact skin. They share no chemical similarity to the histamine liberators so that it is probably safer for the moment to regard them as a separate class.

4. *Histamine releasers, particularly active in vitro. Mongar* and *Schild* have studied a number of substances, using a diffusion technique *in vitro*. Two methods were used; in the first a portion of tissue is suspended in a bath, and the releasing agent diffuses into it, while the histamine diffuses out of it. This has the disadvantage of uncertainty about the concentration of drug in the tissue, and is fairly slow. In a later method, the tissue is fragmented, so that diffusion plays a much smaller part; here the release is quite rapid and the concentration of drug is known, but of course the integrity of the tissue has been broken.

Under these conditions, *Mongar* and *Schild* (1953) have shown that as well as some of the histamine liberators already mentioned, a number of monoamines, especially octylamine and decylamine, are highly active in releasing histamine. Indeed, by the same procedure, *Arunlakshana* (1953) found a number of antihistamines themselves to be histamine-releasers.

It seems to be thought that such histamine-release may be related to a simple "toxic" effect of the drug in question, perhaps mediated by its surface activity on the cells containing histamine. This work, somewhat different in approach from that on the histamine liberators, has not yet been fully reconciled with work on the whole animal. But the writer finds it hard to believe that the effects produced by histamine liberators depend on the same mechanisms these *in vitro* results, for the following reasons. (a) The latter are obviously fairly closely related to the surface activity of the substance in question; yet in the intact cat the injection of a strongly surface-active material may produce obvious effects but only trivial histamine release, whereas the histamine liberators may be highly active in releasing histamine while entirely lacking the immediate depressor effect of surface active drugs. (b) Although octylamine is very active *in vitro*, its effects by intravenous injection *in vivo* are quite unlike those of a histamine-liberator (*Barger* and *Dale*, 1910). (c) It can be claimed that experiments on the intact cat have so far been entirely concordant with human experience; for instance, the activity of compound 48/80 in the cat, in the perfused cat's skin and in the human skin is in all cases extremely high. But octylamine, which *in vitro* is at least as active as 48/80, is far less active in human skin. (d) The *in vitro* results with mepyramine would imply that a dose of 1 milligramme injected into a cat would produce the kind of modified circulatory shock which 1 milligramme of 48/80 produces in the presence of an antihistamine. But in fact such injections of mepyramine and of other antihistamines hardly ever have any depressor action, and indeed are often pressor.

5. *Miscellaneous, large molecules.* There are a number of histamine releasing processes which cannot be fitted readily into any of the categories so far described. Thus dextran and albumen have been known for some time to produce oedema in the rat, such as is now known to be produced by histamine liberators and which is certainly to be attributed to histamine release (*Edlund* et al., 1952); polyvinylpyrrolidone can also do this in the dog (*Halpern* et al., 1953). Yet there is no reason to suppose that prior sensitisation could have taken place in any way. Similarly *Feldberg* and *Schachter* (1952) found that horse serum injected into the perfused cat's skin produced a prompt and vigorous release of histamine in the absence of any sensitisation of the cat. There are too, the interesting studies of *Rocha e Silva* (*Rocha e Silva*, 1952; *Rocha e Silva* and *Aronson*, 1952)

who has revived the old observation that the incubation of agar with guinea-pig serum produces a substance capable of causing circulatory shock in dogs. In all these cases we have a large molecule producing on its first administration to an animal the release of histamine. *Halpern* has suggested that it may be due to some physical property of the molecule.

The Fate of Mobilized Histamine

A rather complex sequence of events takes place when histamine is set free into the tissue spaces and thence released into the blood. The first step is simply that of distribution in the body. If the plasma histamine level is first raised by an infusion of histamine, and the infusion is then stopped, the plasma histamine falls remarkably rapidly (in a few minutes) to a considerably lower level. *Emmelin* (1951) has produced evidence that this fall is due to diffusion into tissues, and not to destruction of histamine. The subsequent slower decline of plasma histamine, however, is due to its actual destruction or take-up by the tissues.

The second stage may be that of selective localization. Thus after the injection of a large dose intravenously into rats, *Rose* and *Browne* (1938) found that very considerable quantities were taken up by the kidney. Other tissues, taking up rather smaller amounts were, liver, lung, lymph glands, intestine and spleen. Similarly *Emmelin* (1951) found that, after injecting histamine into a renal artery, something of the order of 50% of the histamine could be recovered by extracting the kidney; and, further, that almost all tissues could absorb some of the histamine coming to them in the arterial blood.

A particularly interesting possible example of histamine storage by a tissue is suggested by some recent experiments by *Humphrey* and *Toh* (1954), when they showed that dog platelets could take up hydroxytryptamine added to the fluid surrounding them. These workers showed also that dog platelets could not absorb histamine, but they point out that the dog platelets do not contain histamine, although they do contain serotonin. The possibility of histamine uptake by elements in the blood would be much better tested in a species with histamine-rich platelets. This is an important theoretical question, since it would allow the transport of considerable quantities of histamine in an inactive form to a particular area, and

then permit, by their break-up locally, the release of histamine at this point. It has of course been known for a considerable time that platelet trapping may occur in the lungs in a number of anaphylactoid reactions (*Rocha e Silva*, 1952). We may be wise not to think of histamine transport purely in terms of plasma levels.

The third possible fate for histamine is excretion into the intestine. This is almost certainly only a trivial process; but there is no doubt that histamine can appear in the gastric juice and perhaps some small quantities may succeed in traversing the alimentary tract without being absorbed into the body. It is doubtful whether any unique significance should be attached to the appearance of histamine in the stomach contents since other basic substances may also be excreted there. For instance, morphine may appear in the gastric contents without having been taken by mouth.

A fourth fate for histamine is to be excreted as such in the urine. This occurs to a degree which varies considerably with species. *Anrep* and his colleagues (1944), together with a number of subsequent workers, have shown that carnivores excrete little free histamine, herbivores rather large quantities, and animals of a mixed diet, such as man, dog and cat, an intermediate amount. *Adam* and his colleagues (1954) have studied the fate of infused histamine in man, and find that about 1% appears free in the urine. Other workers have obtained similar results.

Associated with free histamine in the urine is the inactive conjugated product acetyl-histamine which also has been isolated and chemically identified in the urine. This acetylation takes place for the most part in the intestine, because histamine given parenterally produces only trivial amounts of conjugated histamine in the urine. The acetylation is performed by the bacteria in the intestine, of which a number capable of this action have been identified (*Urbach*, 1949). The administration of an antibiotic capable of preventing the growth of these organisms can reduce the amount of conjugated histamine excreted (*Wilson*, 1954). So far as can be judged at present, conjugation is not of importance in the fate of histamine released or injected parenterally, although its role in lessening the amount of histamine free in the intestinal lumen may need attention.

It is only recently that precise information has been obtainable about the break down of histamine. *Best* and this colleagues after

the discovery of histaminase (*Best* and *McHenry*, 1930) put forward the speculation that the ring was broken during metabolism, but the position rested at this point for many years.

A lot of work was done on the distribution of histaminase (principally in the gut, liver and kidney, and in the blood in pregnancy); and the development of histaminase-inhibiting drugs made it possible to verify that in fact the action of histamine could be potentiated as that of acetylcholine can be by eserine. Trachea, gut and uterus show such potentiation of response, but not the cat's blood pressure (*Arunlakshana* et al., 1954).

Recently imidazolacetic acid has been isolated in the urine, although only in small amounts, thus substantiating one's belief that the enzyme histaminase was in fact active under normal conditions. But this only appears in significant quantities when fairly large doses of histamine are given, so that the investigators regard it as probably being an intermediary in the metabolic path (*Tabor* et al., 1953). Recently however, the introduction of the radio-isotope ^{14}C into the histamine ring has allowed this problem to be advanced considerably by *Schayer* and his colleagues (1953). When "radio-histamine" is injected, it was first of all found that none appeared in carbon dioxide of the breath; the histamine breakdown is therefore never complete so that either the ring cannot be broken or the fragment formed is resistant to further degradation.

If the urine of the animals was then chromatographed, a very important observation was made. Three peaks of activity were found which have been named numbers I, II and III, in order of increasing mobility in a butanol-ethanol-ammonia solvent. The first and second peaks consist of unidentified substances, while the third consists of histamine or of acetyl-histamine when this is present. The interesting development has been the finding that only one of these peaks is associated with the activity of diamine oxidase, often referred to as histaminase. The evidence for this rests on the use of drugs known to inhibit diamine oxidase, by which it can be shown that the C14 activity is shifted from the first peak to the third (histamine) peak. Figure 15 shows successive stages of such a shift when the diamine oxidase inhibitor Rimifon is given to rats in threshold dose (left) medium dose (centre) and maximally effective dose (right). But it is possible to obtain almost complete obliteration of peak I while leaving II quite unaltered. We have

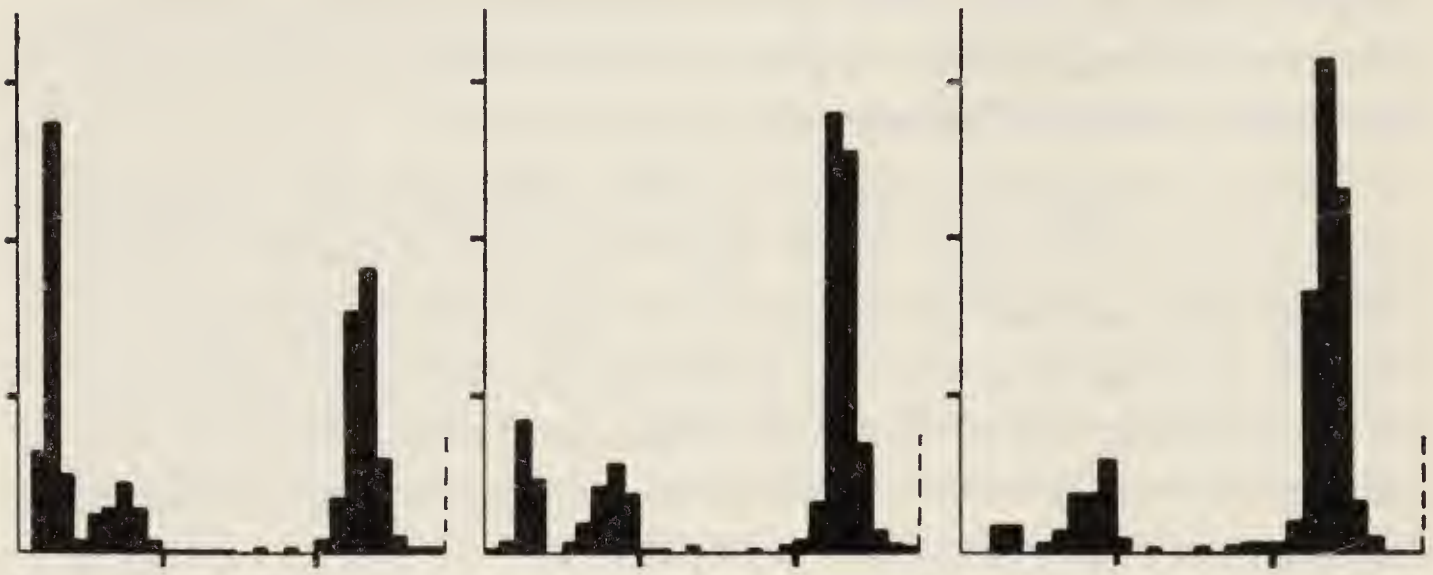


Fig. 15. Radioactivity on paper chromatograms of urine of rats showing effect of various concentrations of Rimifon on the pattern of histamine metabolites. Abscissa, position on paper, each division = 10 cm. Ordinate, per cent of total counts per minute on paper, each division = 10 per cent. Concentrations of Rimifon (micrograms per g. of body weight) 10, 50, and 100 respectively. (From *Schayer* et al., 1953.)

therefore an indication of a second break-down pathway for histamine of quite a different kind, at the head of which stands the enzyme which *Schayer* and his colleagues have chosen to call lucidly (but inelegantly!) "histamine metabolising enzyme number two". This second enzyme can indeed be inhibited by certain drugs which also inhibit diamine oxidase, but these drugs are not specific, and its difference from diamine oxidase is quite clear.

The position is rendered still more interesting and complicated by finding that different species seem to use two pathways to a varying extent. Thus the rat favours the first route most, thus depending to a large extent on its diamine oxidase; the guinea-pig employs both pathways; while the mouse, the hamster and the cat use to progressively greater extent the second route. Unfortunately, there seems no clear indication as to whether man must in this connection be regarded as a rat, guinea-pig or cat; no doubt there has been only a sluggish flow of volunteers to receive injections of ^{14}C histamine!

It is clear that considerable developments can be expected in this direction, particularly when the biochemical identity of the breakdown products in question have been settled. These workers have gone further and studied how far the biochemical pathways can be influenced by hormones or the removal of the adrenals. They failed to obtain any evidence that pregnancy, progesterone, adrenalectomy, or oestradiol would influence the path of histamine

metabolism at all. Cortisone, however, did appear to cause some shift from both the first and second peaks into the third, i. e. it interfered with histamine metabolism, but in some way probably not anti-enzymic, since it affected both peaks equally.

A study of quite a different kind, using similar methods, was also made to test whether it would be possible to influence the manufacture of histamine in the body. There is known to be an enzyme, histamine decarboxylase, in liver and kidney, which forms histamine from its parent aminoacid. A number of inhibitors of this enzyme, including d-catechin, have been described, and these were administered to animals to see whether they could modify the histamine pattern. No change, however, was produced; and it seems that we must wait for a while before we can interfere successfully with histamine synthesis in the body.

Conclusion

Finally, we should consider, briefly, whether any of this recent work offers any real hope of adding to the allergist's therapeutic armoury. I suppose that at present his principal weapons are: (1) the discovery and avoidance of antigen; (2) desensitization; (3) antihistamines. A new attack might go along a number of lines: (a) *Attenuation of the histamine-releasing process*. Although some scanty indications exist that this is experimentally possible with calcium, ether anaesthesia, and perhaps some compounds like phenergan, nothing useful has emerged. Antihistamines in general do not seem to interfere with histamine-release, only with its effects. (b) *The exhaustion of the histamine stores*. As *Feldberg* and *Talesnik* showed, a profound and sustained reduction in skin histamine can be achieved in rats, and such rats become resistant to egg-albumen and to photosensitization. But it has not proved possible to do this to all tissues. Further, the administration of histamine-liberators repeatedly has had its own effects, including that of stomach ulceration and intestinal damage – the former of which, of course, is not treatable by antihistamines, so that a cat became moribund after repeated injections of histamine-liberator. Finally, serious chronic toxic effects have been described for some of the histamine liberators. (c) *The prevention of histamine synthesis*. This field has not been properly explored, and although it was claimed that d-catechin, a known inhibitor of histidine decarboxylase, could protect guinea-

pigs from anaphylactic shock, (as so many things have been claimed to do) a test by *Schayer* and his colleagues for an inhibition by this compound of decarboxylation *in vivo* proved negative. (d) *Acceleration of histamine destruction.* It might be proposed that the more rapid removal of histamine might be achieved. But despite the rather low reputation that histaminase possesses, when one examines the rate at which histamine disappears, it is in fact already rapid. It seems highly unlikely that an acceleration sufficiently great to be useful could be achieved by any means at our disposal. More hopeful, perhaps, might be the cure of some defect in histamine destruction if such occurs, but we still lack any cogent clinical example of this.

The therapeutic dividend to be declared on all this recent work, then, is still small. But one cannot review it without being impressed by the enormous advance in the *precision* of our knowledge about the site, source, release, and fate of histamine – a precision which is bound ultimately to illuminate this still too puzzling body constituent.

Acknowledgements

I am grateful to the Journal of Physiology for permission to reproduce Figures 1, 2, 3, 4, 5, 7, 8, 9, 10 and 13; to the British Journal of Pharmacology for Figures 6, 11 and 12, and to the Journal of Biological Chemistry for Figure 15.

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